



07/22/2022

2022.07.22 Project Skunkworks: Mesilla Valley Cloning Systems

Updates:

2022.11.22 - v_1.3 corrected section 2.2.1

2022.11.22 - v_1.2 corrected typos in section 1.2.2, changed font from Roboto Mono to IBM Plex Mono.

2022.11.22 - v_1.1 corrected a mislabeled homology arm in section 3.1.3.

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Creative Business Concept: A system of plasmids that employ the use of SLiCE based homologous recombination based cloning, TypeIIS and TypeII restriction enzyme based cloning methods. The plasmids are unified by common design language and are cross compatible with each other. These plasmids serve 3 basic applications: 1) cloning non coding DNA for tiered based assembly, 2) for storing a sequence in a plasmid, 3) for expressing a protein sequence either with or without a tag. The system uses his, myc and HA tags for protein purification and epitope labeling.

Chromogenic proteins are used as a selection marker for selecting a colony harboring a specific plasmid in molecular cloning procedures like TA cloning (1), Golden Gate and Golden-Gate-like DNA cloning (2), SLiCE molecular cloning (3), and traditional restriction enzyme/t4 ligase based DNA cloning (4). The plasmid backbone for all RGB vectors is a synthetic **pRadegenBio.pUC+** designed based on a modified pUC18 plasmid with a modular antibiotic selection marker.

1. Red-Green-Blue (RGB) Gate Cloning Plasmids

- 1.1. Cloning plasmids for DNA assembly using TypeIIS restriction enzymes. The system employs the use of 3 distinct cloning vectors ment for assembly using 3 distinct type IIS restriction enzymes. Each plasmid also codes for a specific chromogenic protein (5) designed for selection *E. coli* colony harboring a distinct vector. This system enables a reduction of consumables by providing the ability to combine distinct ligation reactions into one *E. coli* transformation reaction. Colonies obtained from the RGB transformation reactions are screened based on color. Red colonies harbor the BsaI plasmid, green colonies harbor the SapI plasmid and blue colonies harbor the BsmBI plasmid. Labs that spend tens of thousands of dollars USD on LB broth and agar plates can potentially reduce their cost by two thirds. RGB Gate Cloning Plasmids code for a **Kanamycin** resistance selection marker.

- 1.2. The system is specifically designed for simple cloning reactions with one plasmid, tiered based assembly of synthetic DNA from duplexed oligonucleotides and synthetic DNA in various formats to complex braid assemblies.

1.2.1. pRBio.pUC.BsaI.red - a plasmid harboring a red chromogenic protein selection marker and a Multiple Cloning Site MCS containing the following sequence:

BsaI->
 5'-GATTACAGCAATGAGACC NNN NNN NNN GGTCTCACTAACATTAG-3'
 3'-CTAATGTCGTTACTCTGG NNN NNN NNN CCAGAGTTGATTGTAATC-5'
 <-IasB

- 1.2.1.1. Red colored text is BsaI recognition site in opposing directions
- 1.2.1.2. Blue color text is MCU sequence flanked by opposing BsaI sites
- 1.2.1.3. Green colored text depicts the 4 bp 5' overhang retained by the vector backbone after BsaI digest
- 1.2.1.4. BsaI terminal cloning **adaptors** for synthetic DNA construct
 5' GATTACAggtctcAnnnn NNNNNNNN nnnnTgagaccACATTAG 3'
- 1.2.1.5. Primers for adding terminal adapter by PCR
 5' gattacaggtctcannnn - 3'
 5' ctaatgtggtctcannnn -

1.2.2. pRBio.pUC.SapI.green - a plasmid harboring a green chromogenic protein selection marker and a MCS containing the following general characteristics:

SapI->
 5'-GATTACA**ATGCGAAGAGC** NNN NNN NNN **GCTCTTCCTAG**ACATTAG-3'
 3'-CTAATGTTAC**GCTTCTCG** NNN NNN NNN **CGAGAAGGATCTGTAATC**-5'
 <-IpaS

- 1.2.2.1. Green color text is SapI recognition site in opposing directions
- 1.2.2.2. Blue color text is MCU sequence flanked by opposing SapI sites
- 1.2.2.3. Red colored text depicts the 3 bp 5' overhang retained by the vector backbone after SapI digest

1.2.3. pRBio.pUC.SapI.gfp - a plasmid harboring a green fluorescent protein selection marker. This plasmid is meant for use as a destination vector to harbor a cDNA sequence for assembly purposes. The cDNA sequence should start with "TATG" followed by the second codon in the cDNA sequence and end with the codon before the natural stop codon. Digestion of this plasmid should be followed by gel purification. The cDNA fragment is then ligated into the 3 bp overhang sites on a linearized and gel purified **OpSE MCS Plasmid produced by SapI digestion. Please see **OpSE SapI T7 Expression Cloning Adapters (2.2.2)** below for guidance on designing a synthetic DNA fragment for use with this plasmid.**

SapI->
 5' - GATTACAgctcttcTATG nnn nnn nnn TAATCCCGaagagcACATTAG - 3'
 3' - CTAATGTcgagaagataC nnn nnn nnn ATTAGGGcttctcgTGTAAATC - 5'
 <-IpaS

1.2.4. pRBio.pUC.BsmBI.blue - a plasmid harboring a blue chromogenic protein selection marker and a MCS containing the following general characteristics:

BsmBI->

```

5'-GATTACAACCGTgagacg NNNNNNNNNN cgtctcTACTAACATTAG-3'
3'-CTAATGTTGGCActctgc NNNNNNNNNN gcagagATGATTGTAATC-5'
<-IBmsB

```

- 1.2.4.1. Blue color text is BsmBI recognition site in opposing directions
- 1.2.4.2. Red color text is MCU sequence flanked by opposing BsmBI sites
- 1.2.4.3. Green color text depicts the 4bp 5' overhangs retained by the vector backbone after BsmBI digestion
- 1.2.4.4. BsmBI terminal cloning **adaptors** for synthetic DNA construct
5' GATTACAgctctcTnnnn NNNNNNNNN nnnnTgagacgACATTAG 3'
- 1.2.4.5. Primers for adding terminal adapters by PCR
5' gattacacgtctctnnnn 3'
5' ctaatgtcgtctcannnn 3'

2. OpenSource Enzyme (OpSE) Multiple Cloning Site (MCS) Plasmid (OpSE Plasmids)

2.1. Cloning plasmid suite containing the OpSE MCS. The multiple cloning site is designed to contain the restriction enzyme recognition site for Radegen Biotechnology's suite of restriction enzymes based on Open Source enzyme technology. The MCS is adjacent to an expression cassette meant for cloning a coding sequence by SapI digestion for IPTG inducible T7 expression. A synthetic donor plasmid or dsSynthDNA fragment with the SapI digestion adapters can be used. **The cDNA sequence should start with "TATG" followed by the second codon in the cDNA sequence and end with the natural stop codon. A digestion adapter would be designed as follows and can be harbored on a synthetic dsDNA fragment (2.2.2) or as (pRadegenBio.pUC.rbGFP.SapI):**

2.2. OpSE - SapI T7 Site - A cloning site included in the **OpSE Plasmids suite**. This plasmid is specifically designed to construct a T7 IPTG inducible construct from a cDNA sequence in a synthetic DNA format and designed with the **OpSE SapI T7 Expression Cloning Adapters**.

2.2.1. pRBio.OpSE.MCS.amp

```

                    _T7 promoter_-----
5' - tccggcgtag aggatcgaga tcgatctcga tcccgcgaaa ttaatacgac tcactatagg

_lac operator_-----_RBS__
ggaattgtga gcgataaaca attccctct tgaataaatt ttgtttaact ttaagaagga

_
SapI    NotI    NcoI    HindIII  PstI    XbaI    EcoRI
gatatacaTA TGAAGAGCt gcggccgcCC ATGgaagctt atCTGCAGtt tctagaGAAT

EcoRV    SpeI    SapI                    SfiI
TCgatattt ACTAGTttGC TCTTCCCCCG ATTACAggcc AatgTggccGAT TACA - 3'

```

- 2.2.1.1. SfiI is considered a rare cutter with an 8bp recognition sequence and is ideal for plasmid linearization.

2.2.2. OpSE SapI T7 Expression Cloning Adapters

```

5'_Terminal_Adapter_____ 3'_Terminal_Adapter____
5' - GATTACAGCTCTTCAt atg nnn nnn nnn taa tcccCGAAGAGCACATTAG - 3'
3' - CTAATGTCGAGAAGTa tac nnn nnn nnn ttt agggGCTTCTCGTGTAAATC - 5'

```

- 2.2.2.1. Blue color text is the SapI recognition site in opposing directions.
- 2.2.2.2. NNN region in red should consist of cDNA sequence for protein of interest starting at the second codon and ending at the codon before the natural stop codon.
- 2.2.2.3. Green colored text including the nested restriction enzyme recognition site consists of terminal cloning adapters. These terminal adapters can be amended to any sequence for cloning into the SapI site.

3. Ppu λ red.cloning Plasmids

- 3.1. +43 cloning plasmid suite for use in SLiCE based cloning and DNA assembly. Radegen Biotechnology's SLiCE based cloning method is termed Ppu λ red.cloning based on the master mix cloning reagent that will be offered. The suite consists of several plasmids with unique homology sites for tiered DNA assembly or for constructing a final expression plasmid for heterologous protein expression in *E. coli*. Ppu λ red.cloning plasmids code for Tetracycline resistance selection marker. This plasmid suite comes with the +43 6x His tag with an N terminal tag option (3.1.1), a C terminal tag option (3.1.2) and a native expression option (3.1.3)

3.1.1. RadegenBio+43 N - Terminal 7XHis

```

5' - tccggcgtag aggatcgaga tcgatctcga tcccgcgaaa _T7 promoter_____
ttaatacgac tcactatagg

```

```

_lac operator_____ _RBS____
ggaattgtga gcggataaca attccctctc agaaataatt ttgtttaact ttaagagga gatatacat

```

```

_6X_His_Tag_____
ATG CAC CAT CAT CAT CAT CAT TCT TCT GGT CTG GTG CCA CGC GGT TCT GGT ATG AAA
met his his his his his his Ser ser gly leu val pro arg gly ser gly met lys

```

```

_____S-Tag_____ _____
GAA ACC GCT GCT GCT AAA TTC GAA CGC CAG CAC ATG GAC AGC CCA GAT CTG GGT GAA
glu ser ala ala ala lys phe glu arg gln his met asp ser pro asp leu gly glu

```

```

__30 bp homology_arm__ |X|__30 bp homology arm_____
TEV_Cleavage_____
AAC CTG TAC TTC CAG atg TAAgattacagacgacctgcagaatcgctggaaggccggc - 3'

```

Asn leu tyr phe gln|met
 ^ cleavage site

3.1.2. RadegenBio+43 C - Terminal 7X His Tag

```

                                     _T7 promoter_____
5' -   tccggcgtag aggatcgaga tcgatctcga tcccgcgaaa ttaatacgac tcactatagg

                                     5' 30_bp_homology__
_lac operator_____ _RBS__
ggaattgtga gcggataaca attccctct agaaataatt Ttgtttaact ttaagaagga

arm_____|X|_30_bp_homology arm_____
-         _TEV_Cleavage_____
gatatacaATG GAA AAC CTG TAC TTC CAG ATG CCA GAT CTG GGT AAA GAA
      met glu Asn leu tyr phe gln|met pro asp leu gly lys glu
                                   ^

__S-Tag_____
ACC GCT GCT GCT AAA TTC GAA CGC CAG CAC ATG GAC AGC TCT
ser ala ala ala lys phe glu arg gln his met asp ser ser

                                     ____6X_His_Tag_____
CTG GTG CCA CGC GGT TCT TCT GGT CAC CAT CAT CAT CAT CAT
leu val pro arg gly ser gly met his his his his his his

---
TCT TAA gattacagacgacctgcagaatcgctggaaggccggc- 3'
ser *
```

- 3.1.2.1.1. This C-terminal His tag follows the same design language as the N terminal +43 tag. The His tag is distal from the protein to minimize any interference with protein function. An S-tag domain is included in an effort to improve globular protein stability. The tag also contains a TEV cleavage site that leaves a 5 residue tail consisting of N - glu Asn leu tyr phe gln - c.

3.1.2.2. RadegenBio+43 N and C terminal cloning adapters

The above sequence represents the synthetic DNA sequence that should be purchased as synthetic DNA either as dsDNA fragments or as a synthetic plasmid containing the above insert. The variable sequence in the middle should be filled with the cDNA sequence starting at the second codon and ending at the codon before the native stop codon. The sequences below, 2.1.2.2.1 (N terminal) and 2.1.2.2.2 (C terminal) represent an example of synthetic DNA fragment sequences including the homology arms needed for **PpuII** **red.cloning** into the RadegenBio+43 N and C terminal vectors. NNN sequence should consist of a partial cDNA sequence for the protein of interest starting with the second codon to the codon before the stop codon. In 2.1.2.2.3 the blue color text represents a short cDNA sequence consisting of the codon after the start codon (**ATG**) and ending in the codon before the stop codon (**TAA**). This partial cDNA sequence should be divisible by 3 and is typically hundreds of nucleotides in length for most proteins.

3.1.2.2.1. 5' - GATCTGGGTGAAACCTGTACTTCCAGatgNNNNNNNNNNNTAAgattacagacgacctgcagaatcgct - 3'

3.1.2.2.2. 5' - gtttaactttaagaaggagatatacatatgNNNNNNNNNNNGAAACCTGTACTTCCAGATGCCAGATCTG - 3'

3.1.2.2.3. 5' ATG CAC GGT TCT GGT ATT - 3'
met his gyl ser gyl *

3.1.3. RadegenBio+ Native Cloning

```

                    _T7 promoter_____
5' -   tccggcgtag aggatcgaga tcgatctcga tcccgcgaaa ttaatacgac tcactatagg

                                                5' 30 bp homology__
_lac operator_____ _RBS__
ggaattgtga gcggataaca attccctct agaaataatt Ttgtttaact ttaagaagga

arm_____|X|_3' 30 bp homology arm_____
-
gatatacaTATGXTAAgattacagacgacctgcagaatcgctggaaggccggc - 3'
      metX *

```

- 3.1.3.1. This MCS provides the ability to clone a tagless protein by designing the insert synthetic DNA fragment sequence to contain the "5' 30 Homology Arm" and the "3' 30 bp homology arm". The synthetic DNA fragment should consist of the partial cDNA sequence for the protein of interest starting at the second codon and ending one codon upstream of the stop codon. The expression cassette can be bypassed by using the "30 bp homology arm (-T7)" and the "3' 30 bp homology arm" for cloning a non coding sequence.

- 3.1.3.2. Example of a synthetic DNA construct designed for *Ppu*λ **red.cloning** into the RadegenBio Native Cloning Plasmid. The green colored sequence should consist of the cDNA sequence for your protein of interest from the second codon to the codon before the stop codon. Purple sequence = homology arms. Sequence 1 is designed for cloning in frame with the expression cassette. Sequence 2 is designed to bypass the expression cassette.

3.1.3.2.1. Cloning module

```

5' 30 bp homology arm      cDNA      3' 30 bp homology arm
5' - gtttaactttaagaaggagatatacaTATGNNNNNNNNNTAAgattacagacgacctgcagaatcgctg - 3'

```

3.1.3.2.2. Cloning module

```

5' 30 bp homology arm      cDNA      3' 30 bp homology arm
5' - ggatcgagatcgatctcgatcccggaatNNNNNNNNNTAAgattacagacgacctgcagaatcgctg - 3'

```

- 3.2. + (GSG)₂ cloning plasmid suite** for use in SLiCE based cloning and DNA assembly. Radegen Biotechnology's SLiCE based cloning method is termed *Ppu*λ **red.cloning** based on the master mix cloning reagent that will be offered. The suite consists of several plasmids with unique homology sites for constructing a final expression plasmid for heterologous tag fusion protein expression in *E. coli*. *Ppu*λ **red.cloning** plasmids code for **Tetracycline** resistance selection marker. This suite consist of both N and C terminal Myc, HA, and 6xHis tag modified by the **+(GSG)₂** linker between the tag and protein of interest

3.2.1. RadegenBio N Term Myc+ Tag Plasmid - an expression plasmid containing an N terminal Myc-(GSG)₂ tag.

```

                    _T7 promoter_____
5' -   tccggcgtag aggatcgaga tcgatctcga tcccgcgaaa ttaatacgac tcactatagg

_lac operator_____ _RBS__
ggaattgtga gcggataaca attccctct agaaataatt Tgtttaact ttaagaagga

                    5' 30 bp homology_____
-       Myc+_Tag_-----
gatatacat ATG GAA CAG AAA CTG ATC TCT GAA GAA GAC CTG GGT TCT GGT
          met glu gln lys leu ile ser gln gln asp leu gly ser gly

-----|X|_3'_30_bp_-----
-----
GGT TCT GGT X TAAgattacagacgacctgcagaatcgctggaaggccggc - 3'
gly ser gly X *
```

- 3.2.1.1. The RadegenBio N Term Myc+ Tag Plasmid is designed to express a N terminal Myc-(GSG)₂ tagged fusion protein by IPTG induction of the T7 promoter. The cloning plasmid is designed for accepting a synthetic DNA construct consisting of the cDNA sequence starting at the second codon and ending at the codon before the native stop codon. This partial cDNA sequence is then amended with the 5' and 3' homology arms. The modified cDNA fragment is cloned into this plasmid by *Ppu*λ **red.cloning**.
- 3.2.1.2. Example of a synthetic DNA construct designed for *Ppu*λ **red.cloning** into the RadegenBio N Term Myc+ Tag Plasmid. The green colored sequence should consist of the cDNA sequence for your protein of interest from the second codon to the codon before the stop codon. Purple sequence = homology arms.

```

5' 30 bp homology arm      cDNA      3' 30 bp homology arm
5' - TGAAGAAGACCTGGGTTCTGGTGGTCTGGTNNNNNNNNNTAAgattacagacgacctgcagaatcgctg - 3'
```

3.2.2. RadegenBio N Term 6xHis+ Tag Plasmid - an expression plasmid containing an N terminal 6xHis-(GSG)₂ tag.

```

5' - tccggcgtag aggatcgaga tcgatctcga tcccgcgaaa ttaatacgcac tcactatagg
                                     _T7 promoter_____

_lac operator_____ _RBS__
ggaattgtga gcggataaca attccctct agaaataatt Tgtttaact ttaagaagga

                    5' 30 bp homology_____|X|_3'
- His+6x+_Tag_____
gatatacat ATG CAC CAT CAT CAT CAT CAT GGT TCT GGT GGT TCT GGT X TAA
          met his his his his his his gly ser gly Gly ser gly X *

_30_bp_____

gattacagacgacctgcagaatcgctggaaggccggc - 3'

```

3.2.2.1. The RadegenBio N Term 7xHis+ Tag Plasmid is designed to express a N terminal 6XHis-(GSG)₂ tagged fusion protein by IPTG induction of the T7 promoter. The cloning plasmid is designed for accepting a synthetic DNA construct consisting of the cDNA sequence starting at the second codon and ending at the codon before the native stop codon. This partial cDNA sequence is then amended with the 5' and 3' homology arms. The modified cDNA fragment is cloned into this plasmid by *Ppu*λ **red.cloning**.

3.2.2.2. Example of a synthetic DNA construct designed for *Ppu*λ **red.cloning** into the RadegenBio N Term Myc+ Tag Plasmid. The green colored sequence should consist of the cDNA sequence for your protein of interest from the second codon to the codon before the stop codon. Purple sequence = homology arms.

```

5' _30_bp_homology_arm      cDNA      3'_30_bp_homology_arm
5' - TGAAGAAGACCTGGGTTCTGGTGGTCTGGTNNNNNNNNNTAAgattacagacgacctgcagaatcgctg - 3'

```

3.2.3. RadegenBio N Term HA+ Tag Plasmid - an expression plasmid containing an N terminal HA-(GSG)₂ tag.

```

                    _T7 promoter_____
5' -   tccggcgtag aggatcgaga tcgatctcga tcccgcgaaa ttaatacgcac tcactatagg

        _lac operator_____                _RBS__
        ggaattgtga gcggataaca attccctct agaaataatt Tgtttaact ttaagaagga

                                5'_30_bp_homology_____
-      HA+_Tag_____
gatatacat ATG TAC CCG TAC GAC GTT CCG CCG TAC GCT GGT TCT GGT GGT
          met tyr pro tyr asp val pro pro tyr ala gly ser gly gly

-----|X|_3'_30_bp-----
-----
TCT GGT ATG X TAA gattacagacgacctgcagaatcgctggaaggccggc - 3'
ser gly met X *

```

3.2.3.1. The RadegenBio N Term HA+ Tag Plasmid is designed to express a N terminal HA-(GSG)₂ tagged fusion protein by IPTG induction of the T7 promoter. The cloning plasmid is designed for accepting a synthetic DNA construct consisting of the cDNA sequence starting at the second codon and ending at the codon before the native stop codon. This partial cDNA sequence is then amended with the 5' and 3' homology arms. The modified cDNA fragment is cloned into this plasmid by *Ppu*λ **red**.cloning.

3.2.3.2. Example of a synthetic DNA construct designed for *Ppu*λ **red**.cloning into the RadegenBio N Term MA+ Tag Plasmid. The green colored sequence should consist of the cDNA sequence for your protein of interest from the second codon to the codon before the stop codon. Purple sequence = homology arms.

```

        5'_30_bp_homology_arm          cDNA          3'_30_bp_homology_arm
5' - CCGTACGCTGGTTCTGGTGGTTCTGGTATGNNNNNNNNNTAAgattacagacgacctgcagaatcgctg - 3'

```

3.2.4. RadegenBio C Term +Myc Tag Plasmid - an expression plasmid containing a C terminal (GSG)₂- Myc tag.

```

          _30 bp homology arm (-T7)_____
                                     _T7 promoter_____
5' -   tccggcgtag aggatcgaga tcgatctcga tcccgcgaaa ttaatacgac tcactatagg

                                     5' 30 bp homology__
          _lac operator_____                                     _RBS__
          ggaattgtga gcggataaca attccctct agaaataatt Ttgtttaact ttaagaagga

Arm_____| X |_3' 30 bp homology arm_____
          +Myc Tag_____
          gatatacaTATG X GGT TCT GGT GGT TCT GGT ATG GAA CAG AAA CTG ATC TCT GAA GAA
                        gly ser gly gly ser gly met glu gln lys leu ile ser gln gln

          -----
          GAC CTG TCT TAA gattacagacgacctgcagaatcgctggaaggccggc - 3'
          asp leu ser *

```

3.2.4.1. The RadegenBio C Term +Myc Tag Plasmid is designed to express a C terminal (GSG)₂-Myc tagged fusion protein by IPTG induction of the T7 promoter. The cloning plasmid is designed for accepting a synthetic DNA construct consisting of the cDNA sequence starting at the second codon and ending at the codon before the native stop codon. This partial cDNA sequence is then amended with the 5' and 3' homology arms. The modified cDNA fragment is cloned into this plasmid by *Ppu*II **red.cloning**.

3.2.4.2. Example of a synthetic DNA construct designed for *Ppu*II **red.cloning** into the RadegenBio N Term Myc+ Tag Plasmid. The green colored sequence should consist of the cDNA sequence for your protein of interest from the second codon to the codon before the stop codon. Purple sequence = homology arms.

```

          5'_30_bp_homology_arm  cDNA  3'_30_bp_homology_arm
5' - GTTTAACTTTAAGATATACATATGGAAGGANNNNNNNNGTTCTGGTGGTCTGGTATGGAACAGAAA - 3'

```

3.2.5. RadegenBio C Term +6xHis Tag Plasmid - an expression plasmid containing a C terminal (GSG)₂- 6xHis tag.

```

          _30 bp homology arm (-T7)_____
                                     _T7 promoter_____
5' -   tccggcgtag aggatcgaga tcgatctcga tcccgcgaaa ttaatacgcac tcactatagg

                                     5' 30 bp homology__
          _lac operator_____                                     _RBS__
          ggaattgtga gcggataaca attccctct agaaataatt Ttgtttaact ttaagaagga

Arm_____| X |_3' 30 bp homology arm_____
          +Myc Tag_____
          gatatacaTATG X GGT TCT GGT GGT TCT GGT ATG CAC CAT CAT CAT CAT TCT TAA
                        gly ser gly gly ser gly met his his his his his his ser *

          gattacagacgacctgcagaatcgctggaaggccggc - 3'

```

3.2.5.1. The RadegenBio C Term +6xHis Tag Plasmid is designed to express a C terminal (GSG)₂-6xHis tagged fusion protein by IPTG induction of the T7 promoter. The cloning plasmid is designed for accepting a synthetic DNA construct consisting of the cDNA sequence starting at the second codon and ending at the codon before the native stop codon. This partial cDNA sequence is then amended with the 5' and 3' homology arms. The modified cDNA fragment is cloned into this plasmid by *Ppu*II **red.cloning**.

3.2.5.2. Example of a synthetic DNA construct designed for *Ppu*II **red.cloning** into the RadegenBio N Term Myc+ Tag Plasmid. The green colored sequence should consist of the cDNA sequence for your protein of interest from the second codon to the codon before the stop codon. Purple sequence = homology arms.

```

          5'_30_bp_homology_arm      cDNA      3'_30_bp_homology_arm
5' - GTTTAACTTTAAGATATACATATGGAAGGANNNNNNNNGGTTCTGGTGGTTCTGGTATGCACCATCAT

```

3.2.6. RadegenBio C Term +HA Tag Plasmid - an expression plasmid containing a C terminal (GSG)₂- HA tag.

```

          _30 bp homology arm (-T7)_____
                                     _T7 promoter_____
5' -   tccggcgtag aggatcgaga tcgatctcga tcccgcgaaa ttaatacgac tcactatagg

                                     5' 30 bp homology__
          _lac operator_____          _RBS__
          ggaattgtga gcggataaca attccctct agaaataatt Ttgtttaact ttaagaagga

          Arm_____| X |_3' 30 bp homology arm_____
                      +HA Tag_____
          gatatacaTATG X GGT TCT GGT GGT TCT GGT ATG TAC CCG TAC GAC GTT CCG CCG
                      gly ser gly gly ser gly met tyr pro tyr asp val pro pro

          -----
          TAC GCT TAA gattacagacgacctgcagaatcgctggaaggccggc - 3'
          Tyr ala *

```

3.2.6.1. The RadegenBio C Term +HA Tag Plasmid is designed to express a C terminal (GSG)₂-HA tagged fusion protein by IPTG induction of the T7 promoter. The cloning plasmid is designed for accepting a synthetic DNA construct consisting of the cDNA sequence starting at the second codon and ending at the codon before the native stop codon. This partial cDNA sequence is then amended with the 5' and 3' homology arms. The modified cDNA fragment is cloned into this plasmid by *Ppu*λ **red.cloning**.

3.2.6.2. Example of a synthetic DNA construct designed for *Ppu*λ **red.cloning** into the RadegenBio C Term +HA Tag Plasmid. The green colored sequence should consist of the cDNA sequence for your protein of interest from the second codon to the codon before the stop codon. Purple sequence = homology arms.

```

          5'_30_bp_homology_arm      cDNA      3'_30_bp_homology_arm
5' - GTTTAACTTTAAGATATACATATGGAAGGANNNNNNNNGGTTCTGGTGGTCTGGTATGTACCCGTAC

```